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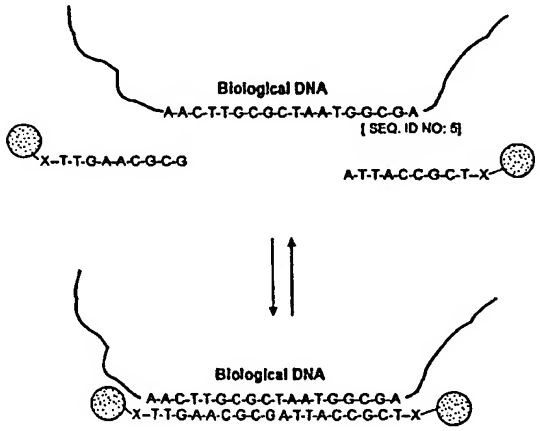
[Continued on next page]

(54) Title: NANOPARTICLES HAVING OLIGONUCLEOTIDES ATTACHED THERETO AND USES THEREFOR



(57) Abstract: The invention provides methods of detecting a nucleic acid. The methods comprise contacting the nucleic acid with one or more types of particles having oligonucleotides attached thereto. In one embodiment of the method, the oligonucleotides are attached to nanoparticles and have sequences complementary to portions of the sequence of the nucleic acid. A detectable change (preferably a color change) is brought about as a result of the hybridization of the oligonucleotideson the nanoparticles to the nucleic acid. The invention also provides compositions and kits comprising particles. The invention further provides methods of synthesizing unique nanoparticle-oligonucleotide conjugates, the conjugates produced by the methods, and methods of using the conjugates. In addition, the invention provides nanomaterials and nanostructures comprising nanoparticles and methods of nanofabrication utilizing nanoparticles. Finally, the invention provides a method of separating a selected nucleic acid from other nucleic acids.

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LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/46418

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, CHEM ABS Data, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LETSINGER R L ET AL: "Use of a steroid cyclic disulfide anchor in constructing gold nanoparticle- oligonucleotide conjugates." BIOCONJUGATE CHEMISTRY, (2000 MAR-APR) 11 (2) 289-91., XP002205939 ---	
A	WO 98 04740 A (UNIV NORTHWESTERN ;MIRKIN CHAD A (US); MUCIC ROBERT C (US); ELGHAN) 5 February 1998 (1998-02-05) ---	
A	WO 99 60169 A (MOLECULAR MACHINES INC) 25 November 1999 (1999-11-25) ---	
A	WO 98 17317 A (SUEDDEUTSCHE KALKSTICKSTOFF ;BAYER ERNST (DE); FRITZ HANS (DE); MA) 30 April 1998 (1998-04-30) ---	
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 July 2003	14/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Molina Galan, E

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/46418

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 25709 A (MEDICAL RES COUNCIL ;HAWKINS TREVOR LEONARD (US)) 23 December 1993 (1993-12-23)	
A	US 5 900 481 A (LOUGH DAVID M ET AL) 4 May 1999 (1999-05-04)	
A	LETSINGER ET AL: "Chemistry of oligonucleotide -gold nanoparticle conjugates" PHOSPHORUS, SULFUR AND SILICON AND THE RELATED ELEMENTS (1999), 144-146, 359-362, XP008019085	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/46418

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-626 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-626 (all partially)

In view of the large number of (independent) claims presently on file and their wording, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible.

An effort was nevertheless made by the ISA to identify a main subject on which a meaningfull search could be performed. The subject apparently most important for the applicant is the preparation of oligonucleotide-nanoparticle conjugates using linkers comprising a steroid residue attached to a cyclic disulfide (cf page 61, line 18 to page 62, line 24). The resulting conjugates have, according to the applicant, an unexpectedly improved sensitivity and surprising stability. This was the only indication enabling the ISA to focus the search within the hundreds of possible embodiments disclosed in the application. The search has therefore been globally performed for the preparation of ligonucleotide-nanoparticle conjugates using linkers comprising a steroid residue attached to a cyclic disulfide, the resulting conjugates and their uses in nucleic acid detection.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/46418

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